Development of SCAR and CAPS Markers Linked to the *Beta* Gene in Tomato

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ABSTRACT

Two previously identified random amplified polymorphic DNA (RAPD) markers, OPAR18$_{1100}$ and UBC792$_{830}$, linked to the *Beta* (*B*) locus in tomato (*Lycopersicon esculentum* Mill.) were cloned and sequenced. Their end sequences were used to design allele specific sequence characterized amplified region (SCAR) primer pairs, SCAR18f/r and SCBC792f/r. Each of these primer pairs amplified a single product of the same size as their respective progenitor RAPD markers, but did not differentiate the two parental genotypes under a variety of polymerase chain reaction (PCR) conditions. Sequence analysis of cloned RAPD and SCAR products revealed an *Rsa*I site mutation in the allele of the high beta-carotene parent in the SCAR18f/r amplified DNA product which was used to develop the codominant SCAR18$_{1067}$ cleaved amplified polymorphic sequence (CAPS) marker. Similarly, a *Hin*fI site mutation in the allele of the high beta-carotene parent in SCBC792f/r amplified DNA product was used to develop the codominant SCBC792$_{779}$ CAPS marker. Sequences from SCBC792f/r amplified DNA products revealed additional single nucleotide polymorphisms between the two parental genotypes. Three such polymorphisms were used to design the nested primer SCBC792f1 located 97 bp internal to the SCBC792f sequence. The SCBC792f1/r primer pair amplified the dominant SCB792$_{682}$ SCAR marker present only in the high beta-carotene parent. Linkage of the CAPS and SCAR markers to *B* was confirmed by means of a population of 144 F$_2$ individuals segregating for *B*. Using introgression line analysis, we mapped the two CAPS and the SCAR markers to the long arm of chromosome 6, consistent with the location of *B* on the classical linkage map of tomato.

EXPRESSION OF THE DOMINANT *Beta* (*B*) locus in tomato produces fruit with high levels of beta-carotene and low levels of lycopene. Beta-carotene is the principal provitamin A carotenoid and is an essential nutrient in the human diet because of its retinoid activity (Omenn et al., 1994). It may also impart health promotive benefits because of its antioxidant properties (Omenn et al., 1994; Paiva and Russell, 1999). Vitamin A deficiency has been described as one of the most serious nutritional disorders of children in the world, especially in developing countries (Sommer, 1997). Epidemiological evidence suggests that increased intake of high beta-carotene containing fruits and vegetables may be associated with a reduced risk of heart disease and certain cancers (Block et al., 1992; Doll, 1990; Omenn et al., 1994; Ziegler, 1989).

Lincoln and Porter (1950) first described the action of the *B* gene in segregants from a cross between the green-fruited wild tomato species, *Lycopersicon hirsutum* Humb. & Bonpl., and a red-fruited *L. esculentum* cultivar. Tomes et al. (1954) subsequently described the influence of a modifier gene, *Mo$_B$*, which influenced the relative proportion of beta-carotene to lycopene. In

breeding for increased beta-carotene content in tomato fruit, traditional methods are based on crosses between cultivated red-pigmented, low beta-carotene genotypes and orange-pigmented, high beta-carotene containing wild species followed by phenotypic selection for adapted high beta-carotene genotypes (Stommel and Haynes, 1994; Tigchelaar and Tomes, 1974; Tomes and Quackenbush, 1958). Molecular markers linked to *B* will be useful in marker-assisted selection (MAS) breeding. By mean of molecular markers linked to a trait of interest, selection can be performed at early seedling stages of development, and true breeding genotypes identified with relative ease.

PCR-based RAPD markers (Welsh and McClelland, 1990; Williams et al., 1990) are useful in MAS because they are simple, rapid and inexpensive, do not require the use of radioactive isotopes, and can be used for analyzing large numbers of samples. However, RAPD markers are sensitive to modifications in PCR reaction conditions, resulting in poor reproducibility (He et al., 1994; Jones et al., 1997; MacPherson et al., 1993; Meunier and Grimont, 1993). Moreover, primers utilized in RAPD analysis usually anneal with multiple sites in different regions of the genome, producing multiple amplification products that often contain repetitive DNA sequences (Paran and Michelmore, 1993). Since RAPD markers are generally dominant, heterozygotes cannot be distinguished from dominant homozygotes (Paran and Michelmore, 1993; Williams et al., 1990).

To overcome the problems associated with RAPD analysis and to improve their utility in MAS application, RAPD markers can be converted into SCAR markers (Paran and Michelmore, 1993). The SCAR markers are generally allele specific, and their amplification is much less sensitive to reaction conditions. A SCAR marker is developed by cloning and sequencing the ends of the amplified RAPD product, generating extended primers specific to the targeted sequences, and amplifying DNA samples under higher stringency conditions (Paran and Michelmore, 1993). They can be developed as dominant markers that generate a single condition of presence or absence, or as potentially codominant markers (Paran and Michelmore, 1993). This strategy has been widely and successfully used to develop markers for various traits in a number of crops (Fang et al., 1997; Gill et al., 1998; Lahogue et al., 1998; Myburg et al., 1998; Ohmori et al., 1996). Despite their promise, monomorphic SCAR products and loss of the initial polymorphism often result (Deng et al., 1997; Maisonneuvu et al., 1994; Ohmori et al., 1996; Paran and Michelmore,

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1993). Several approaches to recover the polymorphisms identified between two parents have been proposed, including optimization of PCR parameters, restriction enzyme digestion of SCAR amplified products to develop CAPS markers, and primer redesign (Akopyanz et al., 1992; Deng et al., 1997; Konieczny and Ausubel, 1993; Maisonneuvu et al., 1994; Moury et al., 2000; Ohmori et al., 1996; Paran and Michelmore, 1993). In the present study, we have explored all of these approaches to develop polymorphic SCAR markers.

Two dominant RAPD markers, OPAR18$_{1100}$ and UBC792$_{830}$, linked to B in tomato were previously identified by means of bulk segregant analysis of an interspecific F$_2$ population developed from a cross of *L. esculentum* $\times$ *L. cheesmanii* f. *minor* (Hook f.) C.H. Mull (Zhang and Stommel, 2000). In the current study, we cloned and characterized these RAPD markers and developed codominant CAPS markers from OPAR18$_{1100}$ and UBC792$_{830}$ and a dominant SCAR marker from UBC792$_{830}$. The codominant and allele specific SCARs can be used to quickly and accurately identify homozygous recessive, heterozygous, and homozygous dominant genotypes at the B locus.

MATERIALS AND METHODS

Plant Material and DNA Extraction

Two parental lines, the high beta-carotene orange-fruited *L. cheesmanii* accession LA317 and the low beta-carotene red-fruited *L. esculentum* cv. Floradade, a segregating F$_2$ population generated from a cross of these lines, and RAPD markers OPAR18$_{1100}$ and UBC792$_{830}$ linked to the B locus, have been described (Zhang and Stommel, 2000). These materials provided a foundation for SCAR and CAPS marker development and analysis. Procedures for DNA extraction and DNA concentration determination were as described in Zhang and Stommel (2000).

Cloning and Sequencing of RAPD Markers

RAPD primers OPAR18 (Operon Technologies Inc., Alameda, CA) and UBC792 (University of British Columbia, Vancouver, BC) were used to amplify genomic DNA of the high beta-carotene parent, *L. cheesmanii* accession LA 317. Amplified fragments were separated on a 1.5% (w/v) low-melting-point agarose gel before being excised and purified by means of the QIAquick Gel Extraction Kit (QIAGEN, Valencia, CA). The purified DNA fragments were ligated and transformed with the pGEM-T Easy Vector System (Promega, Madison, WI). Cloned RAPD fragments were identified via PCR analysis with RAPD primer OPAR18 or UBC792 and the plasmid as template and confirmed by plasmid digestion
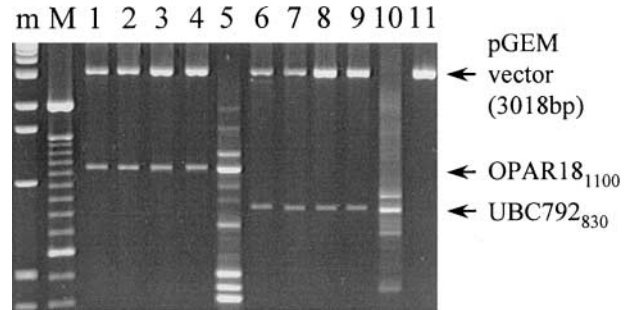
Fig. 1. Cloning of OPAR18$_{1100}$ and UBC792$_{830}$ RAPD markers linked to the B locus by the T/A cloning technique. OPAR18 and UBC792 amplified polymorphic fragments were excised from agarose gels and cloned into pGEM-T Easy vector. The same-sized fragments (indicated by arrows) were recovered from *Eco*RI digested plasmid DNA. Lanes m and M are 1-kbp and 100-bp DNA ladders, respectively. Lanes 1 to 4 and 6 to 9 are individuals of *Eco*RI digested cloned plasmid DNA with OPAR18$_{1100}$ and UBC792$_{830}$ inserts, respectively. Lanes 5 and 10 are RAPD fragments amplified with primers OPAR18 and UBC792, respectively. Lane 11 is a pGEM-T Easy vector.

with *Eco*RI (see Fig. 1). Both 5′ and 3′ ends of cloned fragments were sequenced by means of M13 forward and reverse primers with Perkin-Elmer/Applied Biosystem's AmpliTaq-FS DNA polymerase and Big Dye terminators in an ABI Prism 377 DNA sequencer (Perkin Elmer, Foster City, CA). The DNA sequences of cloned products were determined from four independent clones for each marker and analyzed by DNASIS v2.1 (Hitachi Software, San Bruno, CA). The 'Compare' and the 'Multiple Sequence Alignment' functions were utilized for maximum matching of paired sequences and alignment of multiple sequences from different clones, respectively.
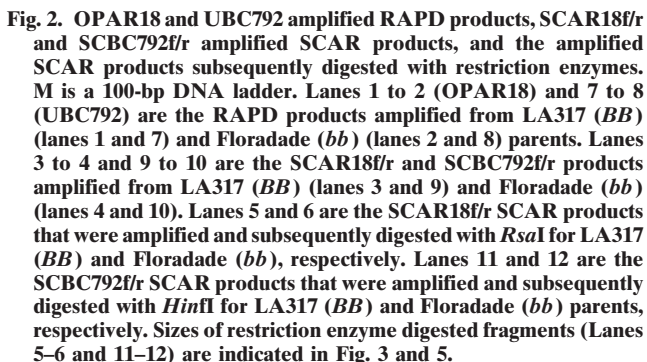
SCAR/CAPS Primer Design and Amplification

On the basis of the sequence of each cloned RAPD product, oligonucleotide primer pairs of 22 or 24 bases were designed for specific amplification of the loci identified by selected RAPD markers. Each primer contained the original 10 bases of the RAPD primer sequence plus the next 12 or 14 internal bases (Table 1). Care was taken to avoid possible primer dimer or secondary structure formation. Primers were synthesized by GIBCO BRL Life Technologies (Gaithersburg, MD). SCAR amplification of LA317 and Floradade genomic DNA was performed in 12.5-μL reaction volumes containing the same components used for RAPD analysis (Zhang and Stommel, 2000), except that the concentration of SCAR primers was 0.4 μM. PCR amplification consisted of 35 cycles of 30 s at 94°C, 30 s at 64 or 60°C (see Table 1), and 75 s at 72°C followed by a final extension of 10 min at 72°C. The amplified products were resolved by electrophoresis on 1.5% (w/v) NuSieve 3:1 agarose gels in 0.5% (v/v) TBE buffer and stained with ethidium bromide.

Table 1. Sequences of 22- or 24-mer oligonucleotide primers for each SCAR locus derived from RAPD markers linked to *B*, optimized annealing temperature, and the type of polymorphism identified in each case.

Locus†	Primer	Sequence (5′–3′)‡ (number of bp)	Annealing temp. (°C)	Polymorphism
SCAR18$_{1067}$	SCAR18f	CTACCGGCACCGGCAAATCAATCA (24)	60–64	Codominant with *Rsa*I digestion
	SCAR18r	CTACCGGCACTTATTCGACTATCA (24)		
SCBC792$_{779}$	SCBC792f	CAACCCACACTAGGCAAGTCGGGT (24)	60	Codominant with HinfI digestion
	SCBC792r	CAACCCACACCCCATTTTTTTA (22)		
SCBC792$_{682}$	SCBC792fl	TGGAAGTCCAAGCCCTAATCAC (22)	60	Dominant
	SCBC792r	CAACCCACACCCCATTTTTTTA (22)		

† The subscript number refers to the size in bp of the amplified SCAR product.
‡ The underlined nucleotides represent the sequence of the original RAPD primers.

Fig. 2. OPAR18 and UBC792 amplified RAPD products, SCAR18f/r and SCBC792f/r amplified SCAR products, and the amplified SCAR products subsequently digested with restriction enzymes. M is a 100-bp DNA ladder. Lanes 1 to 2 (OPAR18) and 7 to 8 (UBC792) are the RAPD products amplified from LA317 (*BB*) (lanes 1 and 7) and Floradade (*bb*) (lanes 2 and 8) parents. Lanes 3 to 4 and 9 to 10 are the SCAR18f/r and SCBC792f/r products amplified from LA317 (*BB*) (lanes 3 and 9) and Floradade (*bb*) (lanes 4 and 10). Lanes 5 and 6 are the SCAR18f/r SCAR products that were amplified and subsequently digested with *Rsa*I for LA317 (*BB*) and Floradade (*bb*), respectively. Lanes 11 and 12 are the SCBC792f/r SCAR products that were amplified and subsequently digested with *Hin*fI for LA317 (*BB*) and Floradade (*bb*) parents, respectively. Sizes of restriction enzyme digested fragments (Lanes 5–6 and 11–12) are indicated in Fig. 3 and 5.

Since the initial SCAR products were monomorphic and did not differentiate the parental genotypes, PCR annealing temperatures of 54 to 72°C and 0.5 to 3× Mg^{++} concentrations were tested to evaluate their effect on amplification products. SCAR products from Floradade were also cloned and sequenced. Sequences from both parental genotypes were analyzed by means of DNASIS as described above. The search function was used to identify restriction site mutations and select appropriate restriction enzymes for CAPS marker development. To digest the SCAR amplified products, 15 U of restriction enzyme and 1× appropriate buffer with or without 1× bovine serum albumin (BSA) based on manufacturer's recommendations were added to the SCAR amplification reactions, and incubated at appropriate temperatures for 4 to 6 h or overnight. Restriction digested PCR products were resolved by electrophoresis on 2% (w/v) NuSieve 3:1 agarose gels.
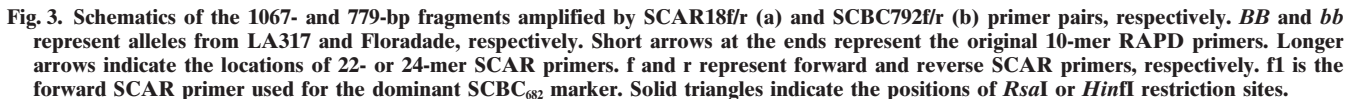
Linkage and Mapping Analysis

Chi-square tests for segregation, linkage analysis and mapping of the developed SCAR and CAPS markers were as described for RAPD markers (Zhang and Stommel, 2000). Recombination frequencies of developed markers were calculated on the basis of the percentage of observed individual recombinants over the total number (144) of F_2 individuals for each marker. Introgression line (IL) analysis (Eshed and Zamir, 1994) was used to map SCAR and CAPS markers on the tomato linkage map. The ILs consisted of 50 *L. esculentum* cv. M82 lines unique for a single chromosomal introgression region from *L. pennellii* LA716. Individual ILs were analyzed for polymorphic SCAR and CAPS products as previously described.

RESULTS

Cloning and Sequencing of OPAR18$_{1100}$ and UBC792$_{830}$

The amplified polymorphic fragment from RAPD primer OPAR18 and UBC792, respectively, were cloned into pGEM-T Easy vectors. Ten transformed colonies from each marker were selected for PCR analysis to confirm recombinant clones. A product of the same size as the original RAPD fragment was amplified for all selected colonies, suggesting that the original RAPD fragment was cloned. For each marker, four individually purified plasmid DNAs from these selected colonies were digested with *Eco*RI to verify the appropriate insert. As expected, the same sized fragment was recovered from each plasmid DNA (Fig. 1). A small difference in size between the cloned fragment and the original RAPD fragment was observed due to the 24 additional bases from the cloning site (Fig. 1). These four cloned plasmid DNAs for each marker, containing the appropriate size insert, were sequenced from both 5′ and 3′ ends. All four individually cloned products from marker OPAR18$_{1100}$ had the same sequence in both forward and reverse directions. However, one of the cloned products from marker UBC792$_{830}$ had a different sequence from the other three cloned products. This variant product was likely not the target product, but rather a comigrating product of similar size and was omitted from further analysis. The full-length sequence for each RAPD marker was obtained by combining

Fig. 3. Schematics of the 1067- and 779-bp fragments amplified by SCAR18f/r (a) and SCBC792f/r (b) primer pairs, respectively. *BB* and *bb* represent alleles from LA317 and Floradade, respectively. Short arrows at the ends represent the original 10-mer RAPD primers. Longer arrows indicate the locations of 22- or 24-mer SCAR primers. f and r represent forward and reverse SCAR primers, respectively. f1 is the forward SCAR primer used for the dominant SCBC$_{682}$ marker. Solid triangles indicate the positions of *Rsa*I or *Hin*fI restriction sites.

forward and reverse sequences and comparing different sequences of individually cloned products. The actual size of the previously identified markers OPAR18$_{1100}$ and UBC792$_{830}$ (Zhang and Stommel, 2000) are 1067 and 779 bp, respectively. These RAPD markers will subsequently be referred to as OPAR18$_{1067}$ and UBC792$_{779}$, respectively.

SCAR/CAPS Analysis

On the basis of the sequence of cloned OPAR18$_{1067}$ and UBC792$_{779}$ products, 22 or 24 bp SCAR primer pairs were synthesized that consisted of the original 10 bases of the RAPD primer sequence plus the next 12 or 14 internal bases (Table 1). Both SCAR18f/r and SCBC792f/r primer pairs amplified a same-sized fragment from LA317 and Floradade parents at a 60°C annealing temperature (Fig. 2). Modified PCR conditions, including various Mg^{++} concentrations and annealing temperatures, did not elicit polymorphic SCAR products between the respective genotypes (data not shown).

To recover the polymorphism detected in RAPD analysis, each of the SCAR18f/r and SCBC792f/r amplified fragments from the Floradade parent were isolated, cloned and sequenced for comparison with corresponding LA317 PCR products. Polymorphic restriction site mutations between the sequences of LA317 (high beta-carotene) and Floradade (low beta-carotene) were identified. In SCAR18f/r amplified products, a single restriction site of *Rsa*I at base 814 and two *Rsa*I sites at bases 675 and 814 were identified from the sequences of LA317 and Floradade parents, respectively (Fig. 2 and 3a). Similarly, a single *Hin*fI restriction site at base 540 and two *Hin*fI sites at 345 and 540 bp were identified from the sequences of LA317 and Floradade parents in SCBC792f/r amplified products, respectively (Fig. 2, 3b, and 4). These restriction site differences enabled development of two codominant CAPS markers, SCAR18$_{1067}$ and SCBC792$_{779}$ (Table 1). When representative high and low beta-carotene F$_2$ individuals were amplified with SCAR18f/r or SCBC792f/r primer pairs and subsequently digested with *Rsa*I or *Hin*fI restriction enzymes, respectively, expected electrophoretic banding patterns were observed (Fig. 5). In high beta-carotene F$_2$ individuals, the heterozygotes (*Bb*) were differentiated from dominant homozygote genotype (*BB*). Dominant homozygotes (*BB*), including the LA317 parent, had two major bands and the recessive homozygotes (*bb*), including the Floradade parent, had three major bands, while heterozygotes (*Bb*) exhibited all four bands for both CAPS markers (Fig. 5).

When the full-length product sequences from the LA317 and Floradade parents were compared, no large insertions or deletions were observed for the SCAR amplified products. Base substitutions and one or a few base insertions or deletions were the only differences detected between the LA317 and Floradade parents in the PCR amplified regions. In SCAR18f/r amplified products, only seven base-substitutions were observed when comparing product sequences of LA317 and Floradade. However, more base substitutions and small in-

Fig. 4. Alignment of SCBC792f/r amplified nucleotide sequences from Floradade (*bb*) and LA317 (*BB*) parents. The original RAPD primer sequences are indicated in bold type and the SCBC792f, f1 and r primers are indicated by arrows. The *Hin*fI restriction sites are indicated in the boxes. The nucleotide differences between *bb* and *BB* are indicated in bold italic type. A period denotes the absence of a nucleotide.

sertions or deletions were observed between the sequences derived from SCBC792f/r amplified products from the LA317 and Floradade genotypes (Fig. 4). A nested 22 bp SCBC792f1 primer, which was located 97 bp down stream from SCBC792f, was designed (Table 1, Fig. 3b, and 4). Amplification of SCBC792f1/r produced a single 682-bp product which was amplified only in the high beta-carotene genotype (*BB* or *Bb*) similar to the original UBC792 RAPD amplification. This presence or absence dominant SCAR marker was designated SCBC792$_{682}$ (Table 1). When high and low beta-carotene F$_2$ individuals were amplified with SCBC792f1/r, a single
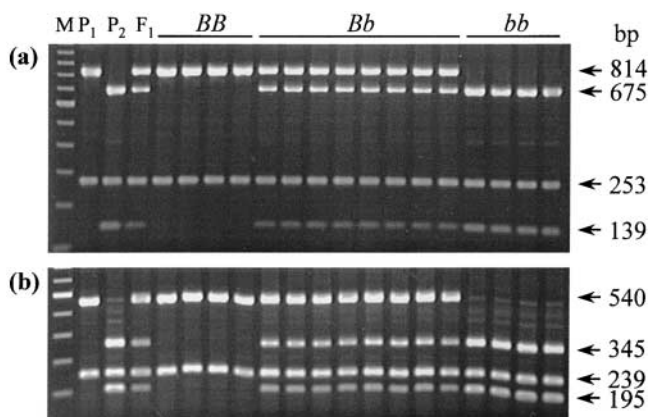
Fig. 5. Two codominant CAPS markers, SCAR18$_{1067}$ (a) and SCBC792$_{779}$ (b), linked to the *B* locus. Monomorphic SCAR18f/r and SCBC792f/r amplified products were digested with *Rsa*I and *Hin*fI, respectively. M is a 100-bp DNA ladder. P$_1$, P$_2$, and F$_1$ are the LA317 (*BB*), Floradade (*bb*) parents, and F$_1$ hybrid (*Bb*), respectively. *BB*, *Bb*, and *bb* are representative F$_2$ individuals of high beta-carotene homozygotes, high beta-carotene heterozygotes, and low beta-carotene homozygotes, respectively.

PCR product of 682 bp was produced in all high beta-carotene (*B_*) but not in low beta-carotene (*bb*) F$_2$ individuals (Fig. 6).

Segregation, Linkage, and Mapping Analyses

Analysis of 144 F$_2$ individuals derived from the cross of *L. esculentum* cv. Floradade × *L. cheesmanii* accession LA317, produced an observed segregation ratio of 107:37 for the presence: absence of SCBC792$_{682}$ ($\chi^2 = 0.04$, $P > 0.95$) which approximated the expected 3:1 segregation ratio, consistent with that observed for fruit pigmentation phenotypes (Table 2). Eleven individuals recombinant for SCBC792$_{682}$ were identified, producing a recombination frequency of 7.6%, which was identical to that previously identified for RAPD marker UBC792$_{779}$ (Zhang and Stommel, 2000). The observed segregation ratios of 32:72:40 and 30:75:39 for high beta-carotene homozygotes (*BB*): high beta-carotene heterozygotes (*Bb*): low beta-carotene homozygotes (*bb*) of SCAR18$_{1067}$ and SCBC792$_{779}$ ($\chi^2 = 3.29$, $P > 0.10$ and $\chi^2 = 5.00$, $P > 0.05$, respectively) approximated the expected 1:2:1
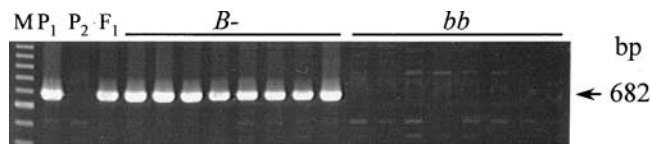

Fig. 6. A dominant SCAR marker, SCBC792$_{682}$, linked to the *B* locus. M is a 100-bp DNA ladder. P$_1$, P$_2$, and F$_1$ are the LA317 (*BB*) and Floradade (*bb*) parents, and the F$_1$ hybrid (*Bb*), respectively. *B_* and *bb* are representative high and low beta-carotene F$_2$ individuals, respectively.

segregation ratio for a codominant gene (Table 2). Six individuals recombinant for SCAR18$_{1067}$ and 9 individuals recombinant for SCBC792$_{779}$ were identified, producing recombination frequencies of 4.2% and 6.3%, respectively which were similar to those obtained for RAPD markers OPAR18$_{1067}$ and UBC792$_{779}$ (4.2% and 7.6%, respectively; Zhang and Stommel, 2000). The highly significant χ^2 for goodness of fit to a 9:3:3:1 ratio expected from two independently segregating loci indicated linkage between the markers and the *B* locus (Table 2). Failure to accept two gene models confirmed linkage between the respective markers and *B* (Table 2). F$_1$ individuals exhibited the orange-fruited high beta-carotene phenotype and, as expected, were scored as heterozygotes using codominant markers (Fig. 5) or as positive for *B*, when using the dominant marker (Fig. 6).

In an effort to map these CAPS and SCAR markers on the tomato molecular linkage map, SCAR18f/r, SCBC792f/r, and SCBC792f1/r primer pairs were used to amplify the DNA samples from a set of 50 introgression lines (ILs) (Eshed and Zamir, 1994; Zhang and Stommel, 2000). SCAR18f/r and SCBC792f/r amplified products were subsequently digested with *Rsa*I and *Hin*fI, respectively. All three markers generated polymorphic products between the IL parents *L. esculentum* cv. M82 and *L. penneillii* LA716 that were equivalent in size and pattern to the polymorphic products produced between the parental lines Floradade and LA317, respectively. Consistent with RAPD marker OPAR18$_{1067}$, the CAPS marker SCAR18$_{1067}$ was mapped within the IL 6–3 introgression region on tomato chromosome 6 between the markers TG279 and TG422, a region spanning approximately 8 cM (Zhang and Stommel, 2000).

Table 2. Segregation and linkage analyses of tomato fruit pigment phenotypes and CAPS or SCAR markers using 144 individuals in an F$_2$ population derived from the cross of *L. esculentum* cv. Floradade × *L. cheesmanii* accession LA 317.

Locus	Observed frequency	Expected ratio	Recombinant		χ^2	P
			No.	%		
Segregation analysis						
Pigmentation	**104:40†**	**3:1**			0.59	>0.30
SCAR18$_{1067}$	**32:72:40‡**	**1:2:1**	6	4.2	3.29	>0.10
SCBC792$_{779}$	**30:75:39‡**	**1:2:1**	9	6.3	3.29	>0.05
SCBC792$_{682}$	**107:37§**	**3.1**	11	7.6	0.04	>0.95
Linkage analysis						
B/SCAR18$_{1067}$	**102:3:37¶**	**9:3:3:1**			446.49	<0.001
B/SCBC792$_{779}$	**100:4:6:34¶**	**9:3:3:1**			381.19	<0.001
B/SCBC792$_{682}$	**100:4:7:33#**	**9:3:3:1**			364.47	<0.001

† Observed fruit pigmentation of F$_2$ individuals (orange, high beta-carotene: red, low beta-carotene).
‡ Observed orange (high beta-carotene) homozygotes (*BB*): orange (high beta-carotene) heterozygotes (*Bb*): red (low beta-carotene) homozygotes (*bb*) of CAPS markers in F$_2$ individuals.
§ Observed presence : absence of the SCAR marker in F$_2$ individuals.
¶ The phenotype order is: orange pigmented fruit and presence of LA317 parental band, orange pigmented fruit and presence of Floradade parental band, red pigmented fruit and presence of LA317 parental band, red pigmented fruit and presence of Floradade parental band.
# The phenotype order is: orange pigmented fruit and presence of the marker, orange pigmented fruit and absence of the marker, red pigmented fruit and presence of the marker, red pigmented fruit and absence of the marker.

Although we were previously unable to map the RAPD marker UBC792$_{779}$, the CAPS and SCAR markers SCBC792$_{779}$ and SCBC792$_{682}$ were successfully mapped to the same location as SCAR18$_{1067}$, between the markers TG279 and TG422.

DISCUSSION

RAPD polymorphisms presumably result from differences in nucleotide sequence at priming sites. In converting RAPD markers to SCARs, Paran and Michelmore (1993) found that six of nine RAPD polymorphisms were derived from mismatches in one or a few nucleotides in the priming sites. These mismatches were tolerated by extended SCAR primers, producing undifferentiated amplification products from both parents. Likewise, our original SCAR primers for the *B* locus each amplified a single monomorphic PCR product in both high and low beta-carotene parental phenotypes. We were unable to detect the polymorphism produced by the original RAPD primers.

When optimizing PCR parameters, modification of annealing temperatures and Mg^{++} concentrations are generally considered to be most effective (Horejsi et al., 1999; Paran and Michelmore, 1993). Increased annealing temperature can eliminate amplification of the allele from one of the parents by taking advantage of differing amounts of mismatches within a primer sequence. By elevating annealing temperature from 60°C to 67°C, Paran and Michelmore (1993) recovered a dominant SCAR marker in lettuce (*Lactuca sativa* L.). However, in converting SCAR markers in citrus [*Poncirus trifoliata* (L.) Raf.], Deng et al. (1997) found that when annealing temperatures were increased from 50 to 66 to 68°C, the polymorphic amplification products identified between two parental lines were not reliably reproduced. Lowering annealing temperature to the predicted optimal melting temperature allowed them to amplify a clear and reproducible length polymorphism. In the present study, a variety of PCR conditions including various annealing temperatures and Mg^{++} concentrations were tested but failed to differentiate parental genotypes with these primers.

Restriction enzyme digestion of monomorphic SCAR products to recover polymorphism between genotypes requires base mutation within a restriction enzyme site. A screening survey of restriction sites using various frequent cutter restriction enzymes can be used. However, this blind screening survey may not be successful when many enzymes must be screened or no such site mutation exists. In attempts to recover SCAR polymorphisms, Fang et al. (1997) screened 20 restriction enzymes and Naqvi et al. (1996) evaluated 11 enzymes, but both failed to detect polymorphism between respective genotypes. To identify the site mutation more efficiently, we utilized sequence information from SCAR amplified fragments from both genotypes and developed two codominant CAPS markers linked to *B*.

SCAR primers can also be redesigned to generate polymorphism without restriction enzyme digestion if sequence divergent regions between respective genotypes can be identified (Deng et al., 1997; Paran and Michelmore, 1993). Single nucleotide polymorphisms (SNPs) between SCAR amplified products from high and low beta-carotene genotypes allowed us to design another primer set which amplified a dominant SCAR without restriction enzyme digestion.

The codominant nature of a SCAR or CAPS marker will increase the mapping efficiency and the power to order loci because of the increased amount of information that can be derived from F$_2$ individuals. Paran and Michelmore (1993) used codominant SCAR markers for high-resolution genetic mapping of the *Dm* region in lettuce. In the present study, the codominant SCBC792$_{779}$ CAPS marker was positioned successfully on the tomato linkage map. In contrast, we were unable to map its RAPD progenitor UBC792$_{779}$ (Zhang and Stommel, 2000). Codominant markers are also useful for genetic studies since the heterozygous genotype can be distinguished from homozygous dominant individuals. Dominant SCARs are efficiently used in a breeding program if a quick plus or minus assay is needed to identify a locus (Gu et al., 1995; Melotto et al., 1996). Since only a single fragment is amplified, post amplification electrophoresis can be eliminated because the PCR products can be detected directly by staining with ethidium bromide in a microtiter plate or by measuring DNA concentration with an ELISA reader or a spectrophotometer (Weeden, 1994). This would decrease the cost and increase the speed of the analysis.

Both RAPDs and SCARs/CAPS may find applications in marker-assisted breeding applications because they are simple, fast and inexpensive, do not require the use of radioactive isotopes, and can be scaled up for analysis of high throughput applications. SCARs have several advantages over RAPD markers in MAS. Because more stringent reaction conditions are used, the SCAR markers are generally more allele specific. The SCAR amplifications are more stable and reliable, and more easily reproduced in different laboratories with various thermal cyclers. Whereas RAPDs are generally dominant markers, codominant SCARs are more useful in high-resolution mapping and genetic studies (Paran and Michelmore, 1993). In the present study, two codominant CAPS markers and one dominant SCAR marker were developed. These markers may be utilized in marker-assisted breeding programs to track the introgression of the *B* gene into breeding lines and develop adapted tomato germplasm with increased beta-carotene content and improved nutritional value.

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